

Innate partnership of *HLA-B* and *KIR3DL1* subtypes against HIV-1

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Allotypes of the natural killer (NK) cell receptor *KIR3DL1* vary in both NK cell expression patterns and inhibitory capacity upon binding to their ligands, *HLA-B* Bw4 molecules, present on target cells. Using a sample size of over 1,500 human immunodeficiency virus (HIV)+ individuals, we show that various distinct allelic combinations of the *KIR3DL1* and *HLA-B* loci significantly and strongly influence both AIDS progression and plasma HIV RNA abundance in a consistent manner. These genetic data correlate very well with previously defined functional differences that distinguish *KIR3DL1* allotypes. The various epistatic effects observed here for common, distinct *KIR3DL1* and *HLA-B* Bw4 combinations are unprecedented with regard to any pair of genetic loci in human disease, and indicate that NK cells may have a critical role in the natural history of HIV infection.

NK cells are critical components of the innate immune system that have direct involvement in the antiviral immune response¹. NK cells are controlled by many activating and inhibitory receptors^{2,3}, including members of the killer cell immunoglobulin-like receptor (KIR) family. Similarly to other NK cell receptors, KIRs are expressed on T cells as well as on NK cells, affirming their role in both innate and adaptive immunity, but they are distinct from other NK cell receptors in that they are exceptionally diverse and rapidly evolving⁴. Each *KIR* locus encodes either an inhibitory or an activating receptor, except for the *KIR3DL1* gene, which encodes one common activating allotype, *KIR3DS1*, and several inhibitory allotypes. The ligands for the inhibitory *KIR3DL1* allotypes are *HLA-B* molecules that contain the Bw4 motif at positions 77–83 (ref. 5), particularly the subset of Bw4 allotypes containing isoleucine at position 80 (Bw4-80I) as opposed to those with threonine (Bw4-80T) at this position^{5–7}. The ligands for *KIR3DS1* are not known, but this molecule shares 97% sequence similarity with *KIR3DL1* allotypes and may recognize a similar or overlapping set of ligands.

KIRs are expressed in a variegated manner on the NK cell population of a given individual⁸: only a certain percentage of NK cells express the product of a given *KIR* gene. Further, mean fluorescence intensity measurements show subtypes of *KIR3DL1* to be expressed at different abundances on NK cells⁹. Recently, it was shown that NK cell inhibitory capacities of individual *KIR3DL1* allotypes are closely linked to their abundances on NK cells and to the percentages of cells expressing these molecules within the NK cell population of a given individual¹⁰. The functional repercussions of variability at the *KIR3DL1* locus provide a logical, operative grouping system for the alleles of this locus: alleles encoding high-expression allotypes (*KIR3DL1**h), low-expression allotypes (*KIR3DL1**l), and no cell surface expression (*004). *KIR3DL1**004 represents an unusual allele as it encodes a protein that is retained within the cell¹¹, a characteristic that may have functional importance. Variation in the affinity of individual *KIR3DL1* allotypes for various *HLA-B* Bw4 allotypes (controlling for expression levels) has also been shown to a limited extent^{6,10}: the *KIR3DL1* high-expression allotypes that have been

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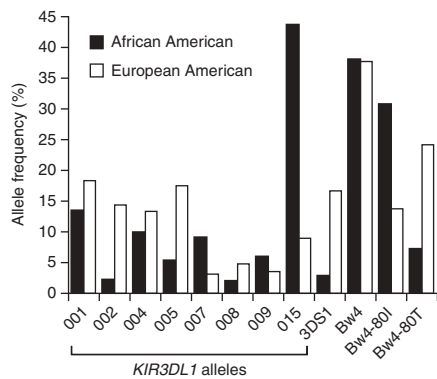


Figure 1 Allele frequency of *KIR3DL1* alleles and *HLA-B Bw4* subtypes. The frequency of each variable is shown for African Americans and European Americans.

tested show higher affinity for Bw4-80I allotypes than for Bw4-80T, which results in greater inhibition through Bw4-80I recognition.

The most protective *HLA* class I alleles in terms of HIV disease progression belong to the group of *HLA-B Bw4* alleles. In particular, two of these *Bw4* alleles, *B*27* and *B*57* (*HLA-B* alleles being represented hereinafter by the allele names standing alone), show the greatest protection in several genetic and functional studies (reviewed in ref. 12), the basis of which stems, at least in part, from the immunodominant HIV peptides recognized by these allotypes in acquired immune responses¹³. However, many of the other members of the *Bw4* group also tend toward protection relative to the alternative group of *HLA-B* alleles, *Bw6* (ref. 14). As individual allotypes belonging to the *Bw4* group recognize distinct peptide motifs¹⁵, it is unlikely that common peptide recognition explains the protection conferred by *Bw4* as a group; rather, overall *Bw4* protection probably has to do with the function of these molecules in the innate immune response as ligands for *KIR3DL1* and/or *KIR3DS1* (*KIR3DL1*;*3DS1*) (ref. 16). Indeed, we have previously shown that the activating *KIR3DS1* allele in combination with *Bw4-80I* associates with protection against HIV disease progression¹⁷, as well as against opportunistic infections in HIV⁺ individuals¹⁸. These data raise the possibility that *KIR3DS1* may bind one or more of the *HLA-B Bw4-80I* allotypes on cells infected with HIV-1, directly mediating effector cell killing of the infected target, a parsimonious model in which NK cell activation renders protection against HIV. Given the functional diversity of the *KIR3DL1* subtypes in terms of the degree of inhibition they confer, it is possible that they may also differentially affect HIV pathogenesis (apart from the protection conferred by *KIR3DS1* + *Bw4-80I*).

Here we tested the effects of inhibitory *KIR3DL1* subtypes in combination with *HLA-B* allelic groups on HIV disease progression and viral load. These data illustrate a primary role for various distinct combinations of *KIR3DL1* and *HLA-Bw4* in the innate immune response against HIV. Of note, the highly expressed, highly inhibitory *KIR3DL1*h* alleles strongly enhance protection conferred by *Bw4-80I* alleles, including *B*57* specifically, an unexpected result given the protective effect of a putatively activating genotype, *KIR3DS1* + *Bw4-80I*, against AIDS progression¹⁷. These seemingly contrasting results can be explained logically by a model in which greater dependency on the expression of specific *KIR3DL1* + *Bw4* receptor-ligand pairs for NK cell inhibition in the resting state (where *KIR3DL1*h* > *KIR3DL1*I*) results in more pronounced NK cell responses when that inhibition is abrogated in the face of infection. This model is con-

sistent with recent studies implicating inhibitory receptors for MHC class I in the education and eventual killing capacity of NK cells^{19–21}.

RESULTS

*KIR3DL1*004* + *Bw4* slows progression to AIDS

We compared the frequencies of each *KIR3DL1* allele in our African American and European American subjects, and the most notable racial differences observed were with *KIR3DL1*015* (a highly expressed allele), which was by far the most common allele in African Americans, and, as reported previously, *KIR3DS1* (ref. 17) (Fig. 1). As was the case for the *KIR3DS1* + *Bw4-80I* effect^{17,18}, the genetic effects described here were consistent across the two main ethnic groups based on analyses stratified by ancestry (see Methods). All statistics provided in this report were derived from analyses stratified by race.

The influence of individual *KIR3DL1* alleles on AIDS progression (see Methods for a description of the AIDS outcomes) was tested in a cohort of 915 HIV⁺ individuals whose date of seroconversion was known within a period of 6 months on average (seroconverters). In order to eliminate confounding by the protective effect of *KIR3DS1* + *Bw4-80I*, individuals with this compound genotype were removed from all analyses described herein. (Our previous study showing protection with this compound genotype¹⁷ was performed in this same cohort). *HLA-B Bw4* (and not *HLA-B Bw6*) allotypes serve as ligands for *KIR3DL1* subtypes^{5,7}. In *Bw6/Bw6* individuals, it does not matter which *KIR3DL1* subtype is present because the ligand is absent, so the *KIR3DL1* molecule is nonfunctional in these individuals. As the *Bw6/Bw6* group does not contain any *KIR3DL1* + *HLA-B* genotype combinations to confound our analyses, we used this group of individuals as a consistent control group for every analysis of *KIR3DL1* subtypes, except as noted otherwise. Using *Bw6/Bw6* as a

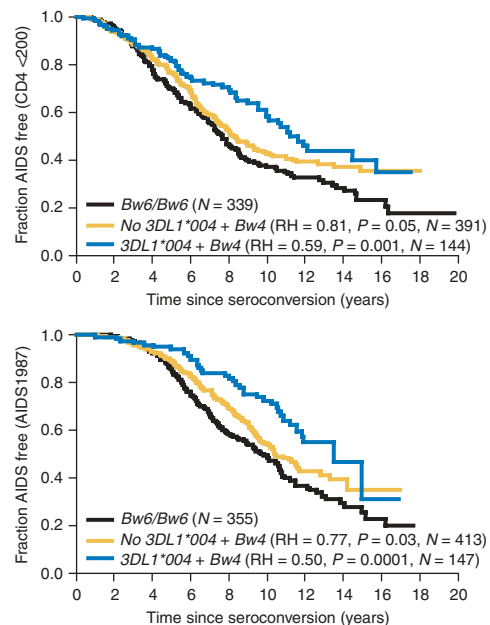


Figure 2 Effect of *KIR3DL1*004* + *HLA-B Bw4* on AIDS progression. Individuals with *KIR3DL1*004* and *Bw4* and those lacking this compound genotype were compared with individuals homozygous for *HLA-Bw6* for two AIDS outcomes. The subjects were seroconverters of all racial groups in combined cohorts. Relative hazard (RH) and significance (*P*) are given for Cox proportional model analyses.

Table 1 Effect of distinct allelic combinations of *KIR3DL1* and *HLA-Bw4* on AIDS progression

Outcome	Genotype	N	RH	95% CI	P value	Test for trend	N	P for trend
CD4 < 200	<i>3DL1</i> *h/*y+Bw4	306	0.70	0.56–0.88	0.002	<i>3DL1</i> *h/*y+Bw4 versus Bw6/Bw6	306	0.002
	<i>3DL1</i> *l/*x+Bw4	182	0.77	0.59–1.00	0.05	<i>3DL1</i> *l/*x+Bw4 versus Bw6/Bw6	182	
							327	
	<i>3DL1</i> *h/*y+Bw4-80I	186	0.61	0.45–0.81	0.001	<i>3DL1</i> *h/*y+Bw4-80I versus Bw6/Bw6	186	0.001
	<i>3DL1</i> *l/*x+Bw4-80I	87	0.89	0.64–1.25	0.51	<i>3DL1</i> *l/*x+Bw4-80I versus Bw6/Bw6	87	
							327	
	<i>3DL1</i> *h/*y+Bw4-80T	148	0.82	0.62–1.08	0.15	<i>3DL1</i> *h/*y+Bw4-80T versus Bw6/Bw6	148	0.01
	<i>3DL1</i> *l/*x+Bw4-80T	113	0.68	0.50–0.94	0.02	<i>3DL1</i> *l/*x+Bw4-80T versus Bw6/Bw6	113	
							327	
							340	
AIDS1987	<i>3DL1</i> *h/*y+Bw4	317	0.66	0.51–0.86	0.002	<i>3DL1</i> *h/*y+Bw4 versus Bw6/Bw6	317	0.001
	<i>3DL1</i> *l/*x+Bw4	194	0.79	0.59–1.06	0.12	<i>3DL1</i> *l/*x+Bw4 versus Bw6/Bw6	194	
							340	
	<i>3DL1</i> *h/*y+Bw4-80I	194	0.56	0.40–0.80	0.001	<i>3DL1</i> *h/*y+Bw4-80I versus Bw6/Bw6	194	0.001
	<i>3DL1</i> *l/*x+Bw4-80I	94	0.79	0.53–1.19	0.26	<i>3DL1</i> *l/*x+Bw4-80I versus Bw6/Bw6	94	
							340	
	<i>3DL1</i> *h/*y+Bw4-80T	152	0.76	0.56–1.03	0.08	<i>3DL1</i> *h/*y+Bw4-80T versus Bw6/Bw6	152	0.05
	<i>3DL1</i> *l/*x+Bw4-80T	119	0.73	0.52–1.04	0.08	<i>3DL1</i> *l/*x+Bw4-80T versus Bw6/Bw6	119	
							340	

P values are based on comparisons between the genotypic variable listed and the Bw6/Bw6 control group. *3DL1**h/*y: *KIR3DL1**h/*h or *KIR3DL1**h/*004; *3DL1**l/*x: *KIR3DL1**l/*l, *KIR3DL1**l/*h or *KIR3DL1**l/*004. CD4, CD4⁺ T-cell count (cells/mm³); CI, confidence interval.

consistent reference group allowed direct comparisons of the degrees of effects conferred by distinct *KIR3DL1* + *Bw4* subtypes. In the presence of *Bw4*, *KIR3DL1**004 showed the most significant protection relative to all other *KIR3DL1* alleles (relative hazard (RH) = 0.50 and 0.59, $P = 0.0001$ and 0.001 ; the first value in each case refers to the AIDS1987 outcome analysis and the second to the CD4⁺ T-cell count < 200 cells/mm³ outcome analysis (see Methods), and this convention will apply wherever two statistics are given; **Fig. 2** and **Supplementary Table 1** online). The q value (the expected proportion of false positives²²) for this association ($q = 0.00005$ and 0.0002) indicates the extreme unlikelihood that this is a false positive discovery (**Supplementary Table 1**). The protective effect of *KIR3DL1**004 was completely dependent on the presence of *Bw4*, as indicated by the lack of any effect in a comparison of individuals with a Bw6/Bw6 genotype who either had *KIR3DL1**004 (Bw6/Bw6 + *KIR3DL1**004; $N = 86$ and 81) or did not (Bw6/Bw6 + no *KIR3DL1**004; $N = 254$ and 246; RH = 1.11 and 0.91, $P = 0.56$ and 0.59). *KIR3DL1**004 is a unique *KIR3DL1* allotype in that it is retained within the cell¹¹, a characteristic that has led to its functionality being called into question. Slow progression to AIDS among individuals carrying *KIR3DL1**004 + *Bw4* raises the possibility that the *KIR3DL1**004 molecule may actually bind Bw4 molecules intracellularly with functionally relevant consequences, a possibility that will require further investigation.

Effects of *KIR3DL1* allelic groups + *Bw4* on AIDS progression

The *Bw4* alleles as a group show protection against AIDS progression¹⁴, and at least part of this protection can be attributed to two independent *KIR* + *HLA* compound genotypes: (i) *KIR3DS1* + *Bw4*-80I (ref. 17) and (ii) *KIR3DL1**004 + *Bw4* (**Supplementary Table 1**; **Fig. 2**). The remaining *KIR3DL1* alleles can be divided into one of two groups: the *KIR3DL1**h (*001, *002, *008, *015, *009) or *KIR3DL1**l (*005, *007) groupings, which were defined previously by *KIR3DL1* expression patterns and, in some cases, corresponding inhibitory capacity^{6,9,10}. Furthermore, in terms of intensity of staining and percentage of NK cells expressing the allotype, *h/*h individuals show significant differences from both *h/*l and *l/*l, but *h/*l and *l/*l do not show any observable difference from one another¹⁰. To test

whether the high-expression alleles differ from the low-expression alleles, individuals were divided into one of two groups: (i) individuals who carried *KIR3DL1**h in the absence of *KIR3DL1**l (i.e., *KIR3DL1**h/*h or *KIR3DL1**h/*004; abbreviated as *KIR3DL1**h/*y, where *y = *h or *004), and (ii) individuals with at least one copy of *KIR3DL1**l (*KIR3DL1**l/*l, *KIR3DL1**l/*h, or *KIR3DL1**l/*004; abbreviated as *KIR3DL1**l/*x, where *x = *l, *h, or *004). (Individuals with

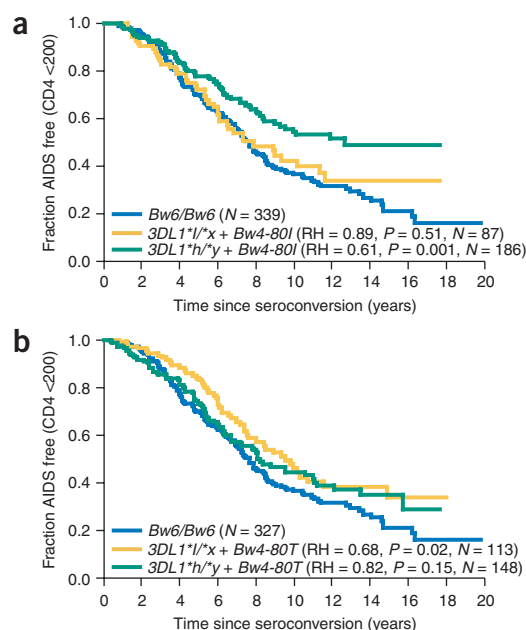


Figure 3 Effect of *KIR3DL1* + *HLA-Bw4* genotypes on progression to CD4⁺ T-cell count < 200 cells/mm³. **(a)** *KIR3DL1* genotypes with *Bw4*-80I. **(b)** *KIR3DL1* genotypes with *Bw4*-80T. Relative hazard (RH) and P values are based on comparisons between the specific genetic variables listed and Bw6/Bw6. The *KIR3DL1**h/*y group includes *KIR3DL1**h/*h and *KIR3DL1**h/*004 individuals, and the *KIR3DL1**l/*x group includes *KIR3DL1**l/*l, *KIR3DL1**l/*h and *KIR3DL1**l/*004 individuals.

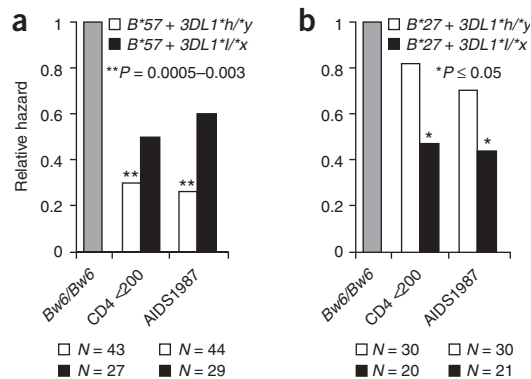


Figure 4 Synergistic influence of specific *HLA-B* allotypes with *KIR3DL1* h* and *KIR3DL1* l* genotypes on progression to two AIDS outcomes. **(a)** *HLA-B*57*; **(b)** *HLA-B*27*. *Bw6/Bw6* individuals were used as the control group in each analysis. The number of individuals in each category is shown below the bars. The *KIR3DL1* h/* y* group includes *KIR3DL1* h/* h* and *KIR3DL1* h/* 004* individuals, and the *KIR3DL1* l/* x* group includes *KIR3DL1* l/* l*, *KIR3DL1* l/* h* and *KIR3DL1* l/* 004* individuals.

*KIR3DL1*004* were not excluded from further analyses because this allele was observed at an equal percentage in the **h* and **l* groups, and the protection conferred by *KIR3DL1*004* + *Bw4* was not significantly different in the presence of **h* versus **l*. Although *KIR3DL1* h/* y* + *Bw4* and *KIR3DL1* l/* x* + *Bw4* were each protective compared with *Bw6/Bw6*, *KIR3DL1* h/* y* showed a more significant effect ($RH = 0.66$ and 0.70 , $P = 0.002$ for *KIR3DL1* h/* y* + *Bw4* and $RH = 0.79$ and 0.77 , $P = 0.12$ and 0.05 for *KIR3DL1* l/* x* + *Bw4*; **Table 1**). Thus, a consistent trend showing more pronounced protection proceeded as follows: *KIR3DL1* h/* y* + *Bw4* > *KIR3DL1* l/* x* + *Bw4* > *Bw6/Bw6* (trend $P = 0.001$ and 0.002).

KIR3DL1 h/* y* + *Bw4-80I* protects against AIDS progression

In studies performed to date, *HLA-B* allotypes with *Bw4-80I* behave as stronger ligands for *KIR3DL1* than do allotypes with *Bw4-80T* (refs. 6,7). Although this stratification had no effect on *KIR3DL1*004* protection (data not shown), the protection conferred by *KIR3DL1* h/* y* + *Bw4* can be attributed primarily to the subset with *KIR3DL1* h/* y* + *Bw4-80I* ($RH = 0.56-0.61$, $P = 0.001$; **Table 1**). In contrast, *KIR3DL1* l/* x* showed no significant protection in the presence of *Bw4-80I*, although this *KIR* + *HLA* combination did tend toward protection compared with the *Bw6/Bw6* group ($RH = 0.79$ and 0.89 ; **Table 1** and **Fig. 3**). Indeed, *KIR3DL1* h/* y* + *Bw4-80I* was borderline significantly more protective than *KIR3DL1* l/* x* + *Bw4-80I* in a stringent, direct comparison of the two groups ($RH = 0.63$ and 0.66 , $P = 0.06$ and 0.04 ; **Supplementary Table 2** online). *KIR3DL1* l/* x* did, however, show some protection against $CD4^+$ T-cell count < 200 cells/mm³ (an earlier outcome than AIDS1987, which includes HIV infection plus an AIDS-defining illness) in the presence of *Bw4-80T* ($RH = 0.68$, $P = 0.02$; **Table 1** and **Fig. 3**). Overall, these data support a high-affinity ligand-receptor interaction between *Bw4-80I* and *KIR3DL1* h*, and raise the possibility of greater affinity of *KIR3DL1* l* allotypes for ligands containing *Bw4-80T* than for those containing *Bw4-80I*. A significant trend for most protective to least protective proceeded as *KIR3DL1* h/* y* + *Bw4-80I* > *KIR3DL1* l/* x* + *Bw4-80I* > *Bw6/Bw6* (trend $P = 0.001$; **Table 1**).

Synergism between specific *Bw4* alleles and *KIR3DL1* h* and **l* *HLA-B*57* and *B*27* are the two most protective alleles in our cohorts of HIV-infected individuals^{23,24}, and a sizeable body of literature has indicated that cytotoxic T lymphocytes restricted by these allotypes serve as the basis for their exceptional ability to control HIV (reviewed in ref. 13). All *B*57* allelic subtypes encode members of the *Bw4-80I* group of allotypes, whereas all of the *B*27* subtypes in our cohorts, with the exception of six subjects with *B*2702*, encoded *Bw4-80T*

Table 2 Effect of combinations of *KIR3DL1* and *HLA-B* on MVL

	Without covariables ^a															P for trend
	MVL < 2,000	MVL > 10,000	OR	95% CI	P value	MVL < 2,000	MVL > 10,000	OR	95% CI	P value	MVL < 2,000	MVL 2,000–10,000	MVL > 10,000			
	N (%)	N (%)				N (%)	N (%)				N (%)	N (%)	N (%)			
<i>Bw6/Bw6</i>	43 (17.3)	205 (82.7)	1.00			43 (17.3)	205 (82.7)	1.00			43 (16.0)	21 (7.8)	205 (76.2)			
<i>3DL1* h/* y</i> + <i>Bw4-80I</i>	63 (47.7)	69 (52.3)	0.24	0.14–0.40	6×10^{-8}	22 (42.3)	30 (57.7)	0.29	0.15–0.59	5×10^{-4}	63 (42.3)	17 (11.4)	69 (46.3)			3×10^{-8}
<i>3DL1* l/* x</i> + <i>Bw4-80I</i>	25 (32.1)	53 (67.9)	0.43	0.23–0.79	0.007	10 (26.3)	28 (73.7)	0.55	0.24–1.28	0.17	25 (29.4)	7 (8.2)	53 (62.4)			0.007
<i>3DL1*004</i> + <i>Bw4</i>	40 (34.2)	77 (65.8)	0.36	0.21–0.62	2×10^{-4}	25 (41.0)	36 (59.0)	0.27	0.14–0.50	5×10^{-5}	40 (32.3)	7 (5.7)	77 (62.1)			4×10^{-4}
<i>3DL1* h/* y</i> + <i>B*57</i>	39 (69.6)	17 (30.4)	0.10	0.05–0.19	7×10^{-11}						39 (62.9)	6 (9.7)	17 (27.4)			4×10^{-11}
<i>3DL1* l/* x</i> + <i>B*57</i>	10 (37.0)	17 (63.0)	0.38	0.15–0.95	0.04						10 (33.3)	3 (10.0)	17 (56.7)			0.02
<i>3DL1* h/* y</i> + <i>B*27</i>	8 (33.3)	16 (66.7)	0.37	0.14–1.00	0.05						8 (25.8)	7 (22.6)	16 (51.6)			0.02
<i>3DL1* l/* x</i> + <i>B*27</i>	10 (47.6)	11 (52.4)	0.22	0.08–0.59	0.003						10 (41.7)	3 (12.5)	11 (45.8)			0.002

P values are based on comparisons between the genotypic variable listed and the *Bw6/Bw6* control group. Genotype symbols are as defined in **Table 1**. CI, confidence interval.

^aFor the *Bw4-80I* groups, all individuals with *KIR3DL1*004*, *B*57* and *B*27* were removed from the analysis. For the *KIR3DL1*004* group, all individuals with *B*57*, *B*27* and *KIR3DL1* h/* y* + *Bw4-80I* were removed from the analysis.

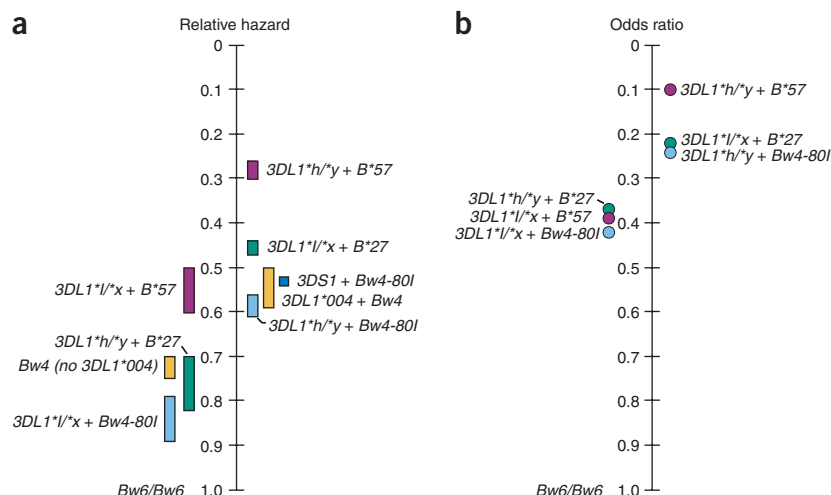


Figure 5 *KIR3DL1 + Bw4* continuum of protection. Genotypes are ordered by degree of protection in terms of (a) disease progression and (b) control of viral load. The most protective genotypes are shown on the right of the vertical scale and the alternative (less protective) genotypes are shown on the left. (a) Relative hazard ranges for the two AIDS outcomes (CD4⁺ T-cell count < 200 cells/mm³ and AIDS1987) relative to the Bw6/Bw6 control group. (b) Odds ratios (dots) of protective genotypes relative to the Bw6/Bw6 control group in terms of their distributions in the < 2,000 versus the > 10,000 MVL groupings. Purple bars and dots, B*57 with *KIR3DL1* genotypes; green bars and dots, B*27 with *KIR3DL1* genotypes; light blue bars and dots, Bw4-80I with *KIR3DL1* genotypes; dark blue bar, Bw4-80I+*KIR3DS1*; yellow bars, Bw4 with or without *KIR3DL1*004*.

allotypes. As *KIR3DL1*h/*y* showed a significant epistatic relationship with Bw4-80I, but not with Bw4-80T, against HIV disease, we tested the hypothesis that *KIR3DL1*h/*y* would enhance the protective effect of B*57, but not B*27 (with the six individuals with B*2702 removed from the analyses). Using Bw6/Bw6 individuals as the control group in each analysis, both the B*57 + *KIR3DL1*h/*y* and the B*57 + *KIR3DL1*l/*x* groups were protective against both AIDS outcomes, but the B*57 effect was enhanced in the presence of *KIR3DL1*h/*y* as compared with *KIR3DL1*l/*x* (RH = 0.26 and 0.30, $P = 0.003$ and 0.0005 for B*57 + *KIR3DL1*h/*y*; RH = 0.60 and 0.50, $P = 0.19$ and 0.06 for B*57 + *KIR3DL1*l/*x*, Fig. 4, Supplementary Table 3 online). The B*57 + *KIR3DL1*h/*y* genotype conferred stronger protection against AIDS progression than any other host genotype identified to date. The progressive effect of B*57 in the presence of distinct *KIR3DL1* groups indicates that the overall protection conferred by B*57 does not stem solely from its function as a cytotoxic T lymphocyte-restricting element in the acquired immune response, but perhaps also as a mediator of innate immunity.

The group of individuals with B*27-80T (B*2705, $N = 60$ and B*2709, $N = 1$), on the other hand, showed borderline significant protection in the presence of *KIR3DL1*l/*x* and only very moderate, nonsignificant protection in the presence of *KIR3DL1*h/*y* (RH = 0.44 and 0.47, $P = 0.05$ and 0.04 for B*27 + *KIR3DL1*l/*x*; RH = 0.70 and 0.82, $P = 0.25$ and 0.46 for B*27 + *KIR3DL1*h/*y*, Fig. 4, Supplementary Table 3). The more prominent protection of B*57 in the presence of *KIR3DL1*h/*y* than in the presence of *KIR3DL1*l/*x* accurately parallels the interaction between *KIR3DL1*h/*y* and Bw4-80I in general, as described above.

Consistent *KIR3DL1 + Bw4* effects on plasma HIV RNA

Several *KIR3DL1 + Bw4* genotypes showed protection against HIV disease progression relative to the control group Bw6/Bw6, the strongest and most conclusive of which included the following:

*KIR3DL1*h/*y + Bw4-80I*, *KIR3DL1*004 + Bw4*, *KIR3DL1*h/*y + B*57*, and to a lesser extent, *KIR3DL1*l/*x + B*27*. We tested the potential effect of these and related genotypes on MVL measurements that were available for 891 subjects from five studies, 508 of whom were unique to these analyses and were not included in the disease progression analyses. Based on their mean viral load (MVL, given throughout in RNA copies per ml plasma), each individual was categorized into one of three groups: (i) MVL < 2,000, (ii) MVL = 2,000–10,000 or (iii) MVL > 10,000. We then determined the frequency of each compound genotype within each of these groupings and compared it with that of Bw6/Bw6.

First we directly compared the two extreme MVL groupings. The relative frequency of *KIR3DL1*h/*y + Bw4-80I* was higher than that of Bw6/Bw6 in the MVL < 2,000 grouping, whereas the opposite was observed in the MVL > 10,000 grouping, and these distributions were significantly different (odds ratio (OR) = 0.24 for *KIR3DL1*h/*y + Bw4-80I*, $P = 6 \times 10^{-8}$, Table 2). Furthermore, they remained significantly different upon removal of individuals positive for B*57,

B*27 and/or *KIR3DL1*004* from the *KIR3DL1*h/*y + Bw4-80I* group (OR = 0.29, $P = 5 \times 10^{-4}$). *KIR3DL1*l/*x + Bw4-80I* also showed significant protection compared with the Bw6/Bw6 group (OR = 0.43, $P = 0.007$; Table 2), but the effect was diminished upon removal of B*57, B*27 and/or *KIR3DL1*004* (OR = 0.55, $P = 0.17$). Further, protection conferred by *KIR3DL1*l/*x + Bw4-80I* was less than that conferred by *KIR3DL1*h/*y + Bw4-80I*, with borderline significance, in a direct comparison of these two Bw4-80I groupings (OR = 0.58, $P = 0.08$; Supplementary Table 2). *KIR3DL1*004 + Bw4* also showed significant protection relative to the Bw6/Bw6 control group (OR = 0.36, $P = 2 \times 10^{-4}$), and the distributions remained significant upon removal of all individuals with B*57, B*27, and/or *KIR3DL1*h/*y + Bw4-80I* from the *KIR3DL1*004 + Bw4* group (OR = 0.27, $P = 5 \times 10^{-5}$; Table 2). These viral load data concur well with the disease progression data and strongly support protective roles for *KIR3DL1*h/*y + Bw4-80I* and *KIR3DL1*004 + Bw4*, even when the samples that overlap between these two groups are removed from the analyses (Supplementary Table 4 online).

The individual effects of B*57 (a Bw4-80I allele) and B*27 (a Bw4-80T allele) on HIV MVL closely followed and emphasized the patterns of association observed in the progression analysis (Fig. 4): in comparison with the Bw6/Bw6 group, B*57 showed exceptionally robust protection in the presence of *KIR3DL1*h/*y* (OR = 0.1, $P = 7 \times 10^{-11}$; Table 2), an effect that was diminished in the presence of *KIR3DL1*l/*x* (OR = 0.38, $P = 0.04$), whereas B*27 was more protective in the presence of *KIR3DL1*l/*x* (OR = 0.22, $P = 0.003$) than in the presence of *KIR3DL1*h/*y* (OR = 0.37, $P = 0.05$). A direct comparison between the B*57 + *KIR3DL1*h/*y* and the B*57 + *KIR3DL1*l/*x* effects on viral load indicated a significantly stronger protective effect of B*57 + *KIR3DL1*h/*y* (OR = 0.23, $P = 0.009$; Supplementary Table 2). These data indicate that *KIR3DL1* allotypes can strongly modulate the protection conferred by B*57 in particular,

and imply an important role for *B*57* (and possibly *B*27*) in the innate immune response against HIV.

In a second set of analyses, the differences in frequency trends across the three viral load divisions (MVL < 2,000, MVL = 2,000–10,000, and MVL > 10,000) were compared between individual *KIR3DL1* + *Bw4* genotypic groups and the *Bw6/Bw6* group (Table 2, far right columns). In each comparison, the relative strength and significance of these differences reflected that observed in the comparisons of the two extreme groups (MVL < 2,000 and MVL > 10,000).

DISCUSSION

The functional consequences of allelic variability at the *KIR3DL1* locus have been studied in greater detail than that of any other single *KIR* gene^{6,10}. In this study, we grouped inhibitory *KIR3DL1* alleles based on distinct functional characteristics and identified differential protective effects of these allelic groups on HIV disease progression and viral load. *KIR3DL1*004* represented the most protective single allele at this locus, and its protection was completely dependent on the presence of *HLA-B* alleles with the *Bw4* motif, the ligands for inhibitory *KIR3DL1* allotypes. We found this result surprising, as the *KIR3DL1*004* molecule is retained within the cell, offering the possibility that it has a null phenotype. The genetic data presented herein dispel this hypothesis and imply a novel intracellular function for *KIR3DL1*004* that directly or indirectly involves the *Bw4* ligand. Alternatively, the *KIR3DL1*004* molecule may be marking (by linkage disequilibrium) another neighboring locus that confers protection in an additive or synergistic manner with *Bw4*.

Previous data have indicated a functional distinction between the strongly inhibitory *KIR3DL1*h* alleles and the weakly inhibitory *KIR3DL1*l* alleles^{9,10}, and based on data presented herein these groupings also have differential effects in the HIV disease process. Overall, a pattern emerged indicating relatively strong protection through *KIR3DL1*h* + *Bw4-80I*, and a weak protective effect through *KIR3DL1*l* + *Bw4-80T*. These data were reinforced by parallel conclusions when the individual *B*57* (*Bw4-80I*) and *B*27* (*Bw4-80T*) alleles were examined and shown to be most protective in the presence of *KIR3DL1*h/y* and *KIR3DL1*l/x*, respectively. Previous functional and genetic studies have also indicated a stronger relationship of some *KIR3DL1* allotypes with *Bw4-80I* than with *Bw4-80T* (refs. 6,7, 10,17,25), but our genetic data imply greater complexity in the receptor-ligand interactions between these molecules. An analysis of MVL data determined from a group of 891 individuals, only 383 of whom overlapped with the 915 individuals used in the disease progression analyses, showed protective effects of the various *KIR3DL1* + *HLA-B* genotypes strongly consistent with those observed in disease progression analyses. Thus, the influence of these genotypes on disease course occurs early after infection, especially for *B*57* + *KIR3DL1*h/y*, and may continue throughout the chronic phase of infection. The protection against AIDS progression conferred by various combinations of *KIR3DL1* + *Bw4* genotypes reported herein represents the first data to support a consequential impact of differential *KIR3DL1* allotypic functions on disease outcome of any sort. There was a continuum of synergistic effects of the *KIR3DL1* and *HLA-B* loci (including *KIR3DS1* + *Bw4-80I*) in order by degree of protection (disease progression, Fig. 5a; MVL, Fig. 5b; see also Table 2 and Supplementary Table 5 online) emphasizing the complexity and strength of protection conferred by combinations of these two loci. There is no other pair of loci evaluated thus far that together shows the diversity in protective effects against AIDS (or any other disease) of that observed with *KIR3DL1;3DS1* and *HLA-Bw4*.

The activating *KIR3DS1* in the presence of *Bw4-80I* associates with slow progression to AIDS¹⁷, lower viral load¹⁸, and protection against opportunistic infections during HIV infection¹⁸. Based on these data, we proposed that NK cell and/or CD8⁺ T cell activation through a *KIR3DS1*–*Bw4-80I* interaction protects against HIV. Further, activating *KIR* genotypes may protect against HIV infection²⁶. This model of effector cell activation leading to protection appears incongruent with the protection conferred by inhibitory *KIR3DL1* subtypes (particularly those that are expressed at high levels and transmit strong inhibitory signals) in the presence of their class I ligands, as shown herein. However, these findings are actually very compatible given our knowledge of the functional development of NK cells and the role of inhibitory receptors in this development. Broadly accepted models of NK cell repertoire development suggest that NK cells continue to acquire inhibitory receptors until sufficient signals are generated to quench autoreactivity through recognition of autologous class I ligands^{27–29}. The resultant population of NK cells utilizes various *KIR* + *HLA* class I pairings that confer various degrees of inhibition to achieve overall nonresponsiveness to self. Therefore, the larger the contribution of a given receptor-ligand pairing to NK cell inhibition under ‘normal’ conditions, the more vigorous an effector cell response will be when the interaction between that inhibitory receptor and its ligand is disrupted. The application of this concept to *KIR3DL1* subtypes is particularly apropos, as there are clear distinctions between *KIR3DL1*h* and *KIR3DL1*l* subtypes in terms of the level of their expression on a per-cell basis⁹, the overall frequency of NK cells expressing these subtypes¹⁰ and the ability of *HLA-Bw4* to increase the frequency of NK cells expressing *KIR3DL1*h* to a significantly greater extent than *KIR3DL1*l*¹⁰. The activation potential of an NK cell population would also be subject to the affinity between the particular *KIR3DL1*–*Bw4* allotypic pair, an effect implied by the synergism between *KIR3DL1*h* and *B*57* and, alternatively, between *KIR3DL1*l* and *B*27* (Table 2 and Fig. 4).

Notably, this concept of greater inhibition during NK cell development ultimately leading to more vigorous activation under appropriate conditions, such as viral infection, is consistent with data implying inhibitory receptor involvement in the developmental regulation of NK cell responsiveness^{8,19–21,30,31}. These data indicate that weak or missing inhibitory signals during NK cell development result in poor (or complete lack of) activating potential by that NK cell, whereas strong inhibitory signals during NK cell development lead to greater potential for NK cell activation upon interaction with an aberrant target cell. Thus, engagement of highly expressed, highly inhibitory *KIR3DL1* allotypes with their ligands may ultimately secure stronger NK cell responses in the event of viral infection, such as HIV, when *KIR3DL1*-mediated inhibition is suspended.

The complexity of *KIR3DL1* is steadily being disentangled by a combination of genetic and functional studies that feed off one another. One allele at this locus, the activating *KIR3DS1*, has been implicated in resistance to both HIV¹⁷ and hepatitis C virus³², and in susceptibility to cervical cancer³³. It is now clear that variation among the inhibitory *KIR3DL1* subset of alleles also has an influence on HIV disease, and dissection of their differential influence is worth investigating in other diseases—such as cervical neoplasia, in which the presence of *Bw4* associates with decreased risk of high-grade cervical lesions and cancer³³. Of the three classical *HLA* class I loci, *HLA-B* is the most rapidly evolving³⁴ and is also the most consequential in the acquired immune response against HIV from both functional and genetic epidemiological perspectives^{23,35}. Notably, however, among *HLA-B* alleles, *Bw4* alleles show greater protection against HIV than *Bw6* alleles, an observation that strongly implicates a defense

mechanism involving *KIR3DL1*, as shown herein. Like *HLA-B*, the *KIR3DL1* locus is also evolving rapidly compared with most other *KIR* loci³⁶, and in our cohorts it is this locus that shows the strongest effects on HIV disease. It is entirely plausible that coordinated coevolution of these two loci^{37,38} is driven by pathogens such as HIV, resulting in a primary influence of specific *HLA-B* + *KIR3DL1* allelic combinations in the innate immune response against this virus.

METHODS

Subjects. We used data from a total of 1,496 HIV⁺ individuals in the studies described. For the disease progression studies, 915 HIV-1-infected subjects for whom the dates of seroconversion were known were derived from four cohorts: the Multicenter AIDS Cohort Study (MACS)³⁹, the Multicenter Hemophilia Cohort Study (MHCS)⁴⁰, the San Francisco City Clinic Cohort (SFCCC)⁴¹ and the AIDS Linked to Intravenous Experience (ALIVE)⁴². For the viral load studies, 891 subjects for whom viral load data was available were obtained from five cohorts: the MACS, the Swiss HIV Cohort (<http://www.shcs.ch>), the Study on the Consequences of Protease Inhibitor Era (SCOPE) cohort⁴³, the Massachusetts General Hospital (MGH) Controller Cohort, and the NIAID long term nonprogressor cohort⁴⁴. There were 383 individuals whose clinical data were used in both the disease progression and the viral load studies. Thus, 508 were used only in the viral load studies and 532 were used only in the disease progression studies. This study was approved by the protocol review office of the US National Cancer Institute institutional review board. Informed consent was obtained at the study sites from all individuals.

HLA genotyping and *KIR3DL1* allelic typing. We performed genotyping of the *HLA* locus following the PCR-SSOP (sequence-specific oligonucleotide probing) typing protocol recommended by the 13th International Histocompatibility Workshop (<http://www.ihwg.org/components/ssop.htm>). For *KIR3DL1* subtyping, polymorphic exons 3, 4, 5, 7, 8 and 9 were selectively amplified in four PCR reactions using locus-specific primers (a separate PCR for each of exons 3, 4 and 5, and a fourth PCR for exons 7, 8 and 9 combined). The PCR products were blotted on nylon membranes and hybridized with a panel of 56 sequence-specific oligonucleotide (SSO) probes designed to detect unique sequence motifs of known *KIR3DL1* alleles (Supplementary Table 6 online). *KIR3DL1* alleles were assigned by the reaction patterns of the SSO probes based on the known *KIR3DL1* sequences. Ambiguous SSO typing results were resolved by sequencing analysis.

Plasma HIV RNA measurements. Viral load measurements (HIV RNA copies per ml plasma in the absence of any drug therapy) determined previously from 891 HIV⁺ individuals were available for analysis. MVL was determined for each individual and categorized into one of three groupings: <2,000, 2,000–10,000, and >10,000. The average number of measurements per person was 7.9 with a range of 1–52 and a total of 7,084 measurements. The ethnic breakdown was as follows: European American (*N* = 685), African American (*N* = 136), other (*N* = 53), unknown (*N* = 17).

Statistical analysis. All statistics provided were derived from analyses stratified by race. Two AIDS-related outcomes were considered in the survival analysis: (i) a CD4⁺ T-cell count of <200 cells/mm³ and (ii) progression to AIDS according to the 1987 definition of the US Centers for Disease Control, which includes HIV infection plus an AIDS-defining illness⁴⁵. In order to eliminate confounding by the previously described protective effect of *KIR3DS1* + *Bw4-80I*, individuals with this compound genotype were removed from all analyses. With the exception of the comparisons listed in Supplementary Table 2, all test variables were compared with those of the *Bw6/Bw6* control group.

The influence of individual and groups of *KIR3DL1* alleles on AIDS progression was tested using Cox model analyses. We divided *KIR3DL1* alleles into high- and low-expressing groups based on the previously defined expression patterns. The high-expressing alleles (*KIR3DL1*^h) in our cohorts were *001, *002, *008, *015 and *009, and the low-expressing alleles (*KIR3DL1*^l) were *005 and *007. *KIR3DL1*^h/*h* individuals show significant differences from both *KIR3DL1*^h/*l* and *l*/*l*, but *KIR3DL1*^h/*l* and *l*/*l* do not show any observable differences from one another in terms of intensity of staining and percentage of NK cells expressing the allotype¹⁰. Furthermore, the high-

expression *KIR3DL1* alleles are more common than the low-expression alleles, resulting in a small *KIR3DL1*^h/*l* genotypic frequency (13% in our entire dataset, 2.7% of whom had *Bw4-80I* and 4.7% of whom had *Bw4-80T*). For these biological and practical reasons, the tests for different effects of high- versus low-expression alleles involved the division of individuals with only high-expression alleles from those with at least one low-expression allele.

We used SAS 9.1 (SAS Institute) for data management and statistical analyses. PROC FREQ was used to compute frequencies on individual variables. PROC LOGISTIC was used for categorical analyses to obtain odds ratios and 95% confidence intervals. PROC LIFETEST and PHREG were used for Kaplan-Meier and Cox model analyses. Statistical significance refers to two-sided *P* values of <0.05. The *q* value shown in Supplementary Table 1 is the false discovery rate. The false discovery rate is the expected proportion of false positives when declaring the significance level equal to *P*; *q* value estimates were calculated for the effect of individual *KIR3DL1* alleles with *HLA-Bw4* on AIDS progression using the *q* value package, v1.1 in the R statistical computing program²².

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

M.C. designed and supervised the project, and prepared the manuscript; M.P.M. performed KIR genotyping and prepared the manuscript; Y.Q. conducted the data analyses; X.G. performed HLA genotyping; E.Y. contributed to KIR genotyping experiments; P.P., S.G.D., D.W.M. provided intellectual input; S.J.O. provided access to samples and clinical data; E.E.B. participated in data analysis; and J.N.M., F.P., S.C., W.L.S., J.P., J.J.G., S.B., G.D.K., A.T., M.C., B.D.W., and S.G.D. provided clinical samples and data. All authors discussed the results and commented on the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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